

# Complementary DNA and deduced amino acid sequences of porcine $\alpha_1$ -microglobulin and bikunin

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Analysis of complementary DNA for porcine  $\alpha_1$ -microglobulin and bikunin indicates that both proteins result from proteolytic processing of a common precursor similar to that found in man. Complete primary structures of these proteins are deduced from the nucleic acid sequence and partially confirmed by peptide sequencing.

Proteinase inhibitor; Inter- $\alpha$ -(trypsin) inhibitor; Kunitz-type structure;  $\alpha_1$ -Microglobulin; Bikunin

## 1. INTRODUCTION

Human  $\alpha_1$ -microglobulin and bikunin are plasma glycoproteins known to result from a common precursor protein by intracellular proteolytic processing [1]. Both these proteins,  $\alpha_1$ -microglobulin as well as bikunin, are also found in plasma protein complexes with IgA [2] and IgG [3], respectively. Furthermore, bikunin is the inhibitor subunit of the inter- $\alpha$ -(trypsin) inhibitor complex whose structure could be determined only recently [4–6].

In severe inflammation and in cancer increased levels of bikunin are observed in serum and urine. We explain these pathophysiological effects by an acute-phase-like increase of bikunin biosynthesis [5]. In order to study such an acute phase reaction in an animal species we first determined the structure of the common precursor protein of porcine  $\alpha_1$ -microglobulin and bikunin.

## 2. MATERIALS AND METHODS

A porcine liver cDNA lambda gt11 library (Clontech Laboratories) was screened with an immunoselected polyclonal rabbit antiserum raised against human bikunin using the ProtoBlot immunoscreening system (Promega Biotec). Plaque-purified DNA was subcloned in plasmid pTZ19R (Pharmacia).

Both strands of the cDNA were entirely sequenced by the dideoxy-chain-termination method, using double-stranded DNA and T7 DNA polymerase (Pharmacia), nested deletions produced by the ExoIII-mung bean nuclease technique, and specific primers.

cDNA sequence data were compiled and analysed using the MicroGenie Sequence Analysis Program, version 5.0 (Beckman).

RNA was isolated from porcine liver by the LiCl/urea method and further purified by affinity chromatography on oligo(dT)-cellulose using a protocol described by Aviv and Leder [7]. Poly(A)-RNA was

analysed by gel electrophoresis (1.4% agarose gel containing 6% formaldehyde) and transferred onto a nylon membrane (Biodyne A, Pall) by capillary blotting. The probe was labeled with <sup>32</sup>P using an oligo-labeling kit (Boehringer Mannheim). After a prehybridization step at 42°C for 3 h, hybridizations were carried out at 42°C overnight with fresh buffer (5 SSC, 10 Denhardt, 50% formamide and sonicated salmon sperm DNA at a concentration of 250  $\mu$ g/ml [1 SSC: 0.15 M NaCl/0.015 M sodium citrate (pH 7); 1 Denhardt: bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.02% each]). Filters were washed at high stringency (0.1 SSC, 0.1% SDS, 65°C). Autoradiography was performed overnight with Kodak X-Omat AR film at –70°C using a Dupont amplifier screen.

Porcine bikunin was isolated, reduced and carboxymethylated as previously described [8,9]. Tryptic peptides either were purified as described in [8,9] or subjected directly to subsequent HPLC runs on a Vydac C<sub>18</sub>-column (50 min; 0.8 ml/min), firstly using a linear gradient (0–100% B) of buffers A (50 mM ammonium acetate pH 6.0) and B (30% A; 70% acetonitrile; pH 6.0), secondly using a linear gradient (0–70% D) of buffers C (0.1% trifluoroacetic acid) and D (20% C; 80% acetonitrile). Peptides were detected at 210 nm and/or 280 nm. Amino acid sequences were determined by solid-phase Edman degradation.

## 3. RESULTS AND DISCUSSION

We obtained a single clone from immunological screening of about 40 000 recombinants of a porcine liver cDNA library. The 1.3 kb cDNA (Fig. 1) detects a prominent band of 1.4 kb and an additional species of 1.5 kb on northern blots of porcine liver poly(A)-RNA (Fig. 2 2). Therefore, we used the cloned cDNA as a probe for a second screening by plaque hybridization. However, all five additional clones contained significantly shorter inserts.

We verified the identity of the porcine  $\alpha_1$ -microglobulin-bikunin clone by comparison with amino acid sequences of peptides isolated from tryptic digests of porcine bikunin (Fig. 1, underlined amino acids) and with the human  $\alpha_1$ -microglobulin-bikunin clone [1].

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GCGGTGAGCGCCAGCCCTGTGCTGACATTGCCAATGACATCCAGGTGCAGGAGAACTTCGACCTGTCTAGGATCTACGGGAAATGGTTC 90
A V S A | S P V L T L P N D I Q V Q E N F D L S R I Y G K W F 26
      -1 microglobulin
CACGTGGCCGTGGGCTCCACCTGCCCTGGCTGAAGAGGTTCAAGGACAAGATGACGATGGGCACGCTGATGCTGGGAGAGGGGGCGACG 180
H V A V G S T C P W L K R F K D K M T M G T L M L G E G A T 56

GAGAGGGAGATCAGCGTGACCAAGACTCACCGGAGGAAAGGTATCTGTGAGGTGATCTCTGGGGCTTATGAGAAAAACAAGCACTGATGGA 270
E R E I S V T K T H R R K G I C E V I S G A Y E K T S T D G 86

AAGTTCCTCTATCACAATCCAAATGGAACATCACCATGGAGTCCTATGTGGTCCACCAACTATGATGAGTATGCCATATTCTGACC 360
K F L Y H K S K W N I T M E S Y V V H T N Y D E Y A I F L T 116

AAGAAGTTCAGCCGCGCCACGGACCCACCTTACTGCCAAGCTCTACGGGCGGGAGCCGAGCTTCGGGAAAGCCTGCTGGAGGAGTTC 450
K K F S R R H G P T L T A K L Y G R E P Q L R E S L L E E F 146

AGGGAGGTTGCCCTGGGCGTGGGCATCCCGGAGGACTCCATCTTTACGATGCCCGACAGAGGGGAGTGTGCTCCCTGGGAGCAGGAGCCT 540
R E V A L G V G I P E D S I F T M P D R G E C V P G E Q E P 176

GAGCCACCTACTCTCAAGAGCCCGCGGGCCGTGCTGCCCCAGGAAGGAGGATCAGGAGCTGGACAACCAGTAGCAGATTTTCAGC 630
E P T L L S R | A R R | A V L P Q E E E G S G A G Q P V A D F S 206
      microglobulin | bikunin
AAGAAAGAAGATTCTGCCAGCTGGGCTACTCCCAAGGCCCTTGCTGGGCATGATCAAGAGGTATTTCTATAATGGCTCATCCATGGCC 720
K K E D S C Q L G Y S Q G P C L G M I K R Y F Y N G S S M A 236

TGCAGACCTTCCACTATGGTGGCTGCATGGGGAACGGCAATAACTTCGTCTCGGAGAAGGAGTGTCTGCAGACCTGCCGGACTGTGGAG 810
C E T F H Y G G C M G N G N N F V S E K E C L Q T C R T V E 266
                        (Q) (S)
GCCTGCAGTCTCCCATCGTCTCCGGCCCTGCCGAGGTTTTTTCAGCTCTGGGCGTTGATGCCGTGCAGGGGAAGTGTGTCTCTTC 900
A C S L P I V S G P C R G F F Q L W A F D A V Q G K C V L F 296
      (Q) (A) (I)(R) (A)
AACTATGGGGCTGCCAGGGCAACGGCAACCAGTCTCTACTCGGAGAAGGAGTGCAAAGAGTACTGCGGCGTCCCGGTGAAGAGGATGAA 990
N Y G G C Q G N G N Q F Y S E K E C K E Y C G V P G E E D E 326
      (K) (Q)
GAGCTGCTGCGCTCCTCCAAGTACCAGCCCGCAGGCCACAGGGCAGCAGGAGGGCCAGCGCCAGCGCTGCTGGTGGCCCATGGCAGG 1080
E L L R S S N 333

TTCCAATAAAACCAATCGTAGCCTCCTGAAATCCACGTCCTGACTGTTTCATCATTAAAGTGAATGAGATGGGGGAGGGGAGGGGGG 1170
ACAAGCTGGGGTGGGGCCGAGTAACCCAGCATCCCAGAAGTGAATAATGTCTGTGTTGAAATGAATAGAACTCTCCTCCATACG 1260
TGAATTTGGCTATGCAAAATTATGAACATAATCACCTTCTGTATAAATGAAAAAAAAAAAAAAAAAAAA 1331

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Fig. 1. Nucleotide sequence and deduced amino acid sequence (below) of cloned porcine  $\alpha_1$ -microglobulin-bikunin cDNA. Deduced amino acid sequences that match those obtained from purified peptides of bikunin are underlined. Residues shown in brackets are taken from the literature [8]. Nucleic acid sequences underlined represent polyadenylation signals.

The deduced porcine  $\alpha_1$ -microglobulin-bikunin sequence extends for 337 amino acids. It is divided into four regions, corresponding to the domains defined by proteolytic processing of the primary translation product. Four C-terminal residues of the signal peptide sequence are encoded on the 1.3 kb cDNA (Figs 1,3). Cleavage of the signal peptide probably occurs at the same site as found in the human precursor protein [1] (Figs 1,3). This would lead to an N-terminal sequence which is also found in rat and rabbit  $\alpha_1$ -microglobulin [10]. Further proteolytic processing occurs at Arg-186 and probably also at Arg-183 releasing the putative linker peptide A-R-R (V-R-R in human). Porcine  $\alpha_1$ -microglobulin therefore would extend to residue 183

(Figs 1,3). The porcine bikunin sequence begins at residue 187 and terminates at residue 333 (Figs 1,4). Both termini were described earlier [8].

Human and porcine sequences of both proteins,  $\alpha_1$ -microglobulin and bikunin, are highly conserved between the species (79% and 83%, respectively). Glycosylation of human as well as porcine bikunin occurs at Ser-10 and Asn-45. In porcine  $\alpha_1$ -microglobulin the glycosylation site (N-X-S/T) at Asn-96 is conserved. The cysteine residues of both proteins are also strictly conserved. In bikunins, cysteine residues are responsible for the typical Kunitz-type structure of this proteinase inhibitor that is also known as an endothelial cell growth factor [11]. In the  $\alpha_2$ -globulin





cDNA (Figs 1,6). However, residues 1314–1331 were clearly identified as a poly(A) tail when the 3'-untranslated region was compared to the corresponding human genomic sequence (Vetr, H. and Gebhard, W., manuscript in preparation) (Fig. 6). Obviously, in the 1.3 kb porcine cDNA, residues downstream position 1113 (broken vertical bar in Fig. 6) represent genomic DNA. The presence of two mRNA species in northern blots of porcine liver RNA therefore may reflect improper polyadenylation rather than transcription from different genes and, therefore, should not complicate studies of  $\alpha_1$ -microglobulin-bikunin biosynthesis in the acute phase.

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